# Synthesis, Crystal structure determination, biological screening and docking studies of $N^1$-substituted derivatives of 2,3-dihydroquinazolin-4(*1H*)-one as inhibitors of cholinesterases

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**Abstract:**

Pursuing the strategy of developing potent AChE inhibitors, we attempted to carry out the $N^1$-substitution of 2,3-dihydroquinazolin-4(1*H*)-one core. A set of 32 *N*-alkylated/benzylated quinazoline derivatives were synthesized, characterized and evaluated for their inhibition against cholinesterases. N-alkylation of the series of the compounds reported previously (N-unsubstituted) resulted in improved activity. All the compounds showed inhibition of both enzymes in the micromolar to submicromolar range. Structure activity relationship (SAR) of the 32 derivatives showed that N-benzylated compounds possess good activity than N-alkylated compounds. N-benzylated compounds **2ad** and **2af** were found very active with their $IC_{50}$ values toward AChE in submicromolar range (0.8 μM and 0.6 μM respectively). Binding modes of the synthesized compounds were explored by using GOLD (Genetic Optimization for Ligand Docking) suit v5.4.1. Computational predictions of ADMET studies reveal that all the compounds have good pharmacokinetic properties with no AMES toxicity and carcinogenicity. Moreover, all the compounds are predicted to be absorbed in human intestine and also have the ability to cross blood brain barrier. Overall, the synthesized compounds have established a structural foundation for the design of new inhibitors of cholinesterase.

**Key Words:** N-alkylated-2,3-dihydroquinazolin-4(1H)-one; Cholinesterases; Alzheimer's disease; Benzylation; Computational studies

## 1. Introduction

The cholinesterases consisting of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) belongs to serine hydrolases enzyme's family, which catalyzes the hydrolysis of the acetylcholine, neurotransmitter and subsequently facilitates termination of the nerve impulse in cholinergic synapses [1]. AChE is part of most tissues, but mostly found in autonomic ganglia, neuromuscular junctions, red blood cell membranes and brain cholinergic synapses [2, 3]. AChE reduces cholinergic neurotransmission in the brain thus having significant role in cognitive impairment and memory associated with Alzheimer disease (AD) [4, 5]. BChE is produced in the liver and commonly found not only in serum and glial cells, but also in smooth muscle cells, adipose tissue, intestine and white matter of the brain and neurons [6]. Various types of physiological processes, like hydrolysis of choline and non-choline esters, succinylcholine, acetylcholine and aspirin[9] have been associated with BChE [7-9]. Thus its activity plays an important role in anesthesia, drug abuse and neurotransmission. Due to the critical role of cholinesterases in maintaining and controlling several important physiological processes in human body, several disorders and diseases are associated with their activity and thus their functions and inhibition is becoming central targets in drug discovery research. Besides clinical use of cholinesterase inhibitors in senile dementia, ataxia, myasthenia gravis, Parkinson's disease and AD], their use in the management of several other conditions like type 2 diabetes and chronic pain may also be beneficial [10-14]. Inhibition of both AChE and BChE enzymes present in neurotic plaques and neurofibrillary tangles will increase ACh level that can interact with neuronal receptor [15]. So far four cholinesterase inhibitors namely tacrine (**1**), rivastigmine (**2**), donepezil (3) and galantamine (**4**) have been licensed for symptomatic treatment of AD (**Figure 1a**). All of these have different pharmacological and pharmacokinetic profiles but all of

them increase ACh level in the brain[16]. Other than this, researchers have developed multiple natural or synthetic origin cholinesterase inhibitors (ChEIs) [17,18].

Although anti-ChE moieties show a wide range of chemical diversity, generally different heterocyclic compound and their derivatives have shown potent inhibitory activity against AChE and BChE enzymes[19- 24]. Amongst heterocyclic moieties, *N*-heterocyclic compounds have the potential to form hydrophobic interaction and nitrogen atom has the ability to donate or accept hydrogen atom and thus form strong hydrogen bonds. Nitrogen atom also shows dipole–dipole or ion–dipole interactions with amino acids in the enzyme gorge [25]. Various nitrogen containing heterocyclic compounds have shown good to excellent inhibition against cholinesterases. These heterocyclic compounds include imidazolidines, oxazolidines and benzoxazoles analogues, 2,3-dihydro-1*H*-cyclopenta[*b*]quinoline derivatives, homo-and heterodimers obtained by coupling of suitable bioactive molecules or existing therapeutics (tacrine, donepezil, galantamine, memantine). [26-30]. To combat the multifactorial nature of AD, a variety of versatile tacrine-related multi-target drugs have gain immense attention. These multi-target tacrine hybrids have shown excellent inhibition due to its pharmacophoric features especially π-π interactions with Trp84 [31-35]. The structures of some tacrine hybrids are shown in **Figure 1b**. Recently, our research group reported the identification of dihydropyrimidines (**4-5**, **Figure 1c**) and 2,3-dihydroquinazolin-4(*1H*)-one derivative (**6**, **Figure 1c**) as inhibitors of cholinesterases [22, 36].

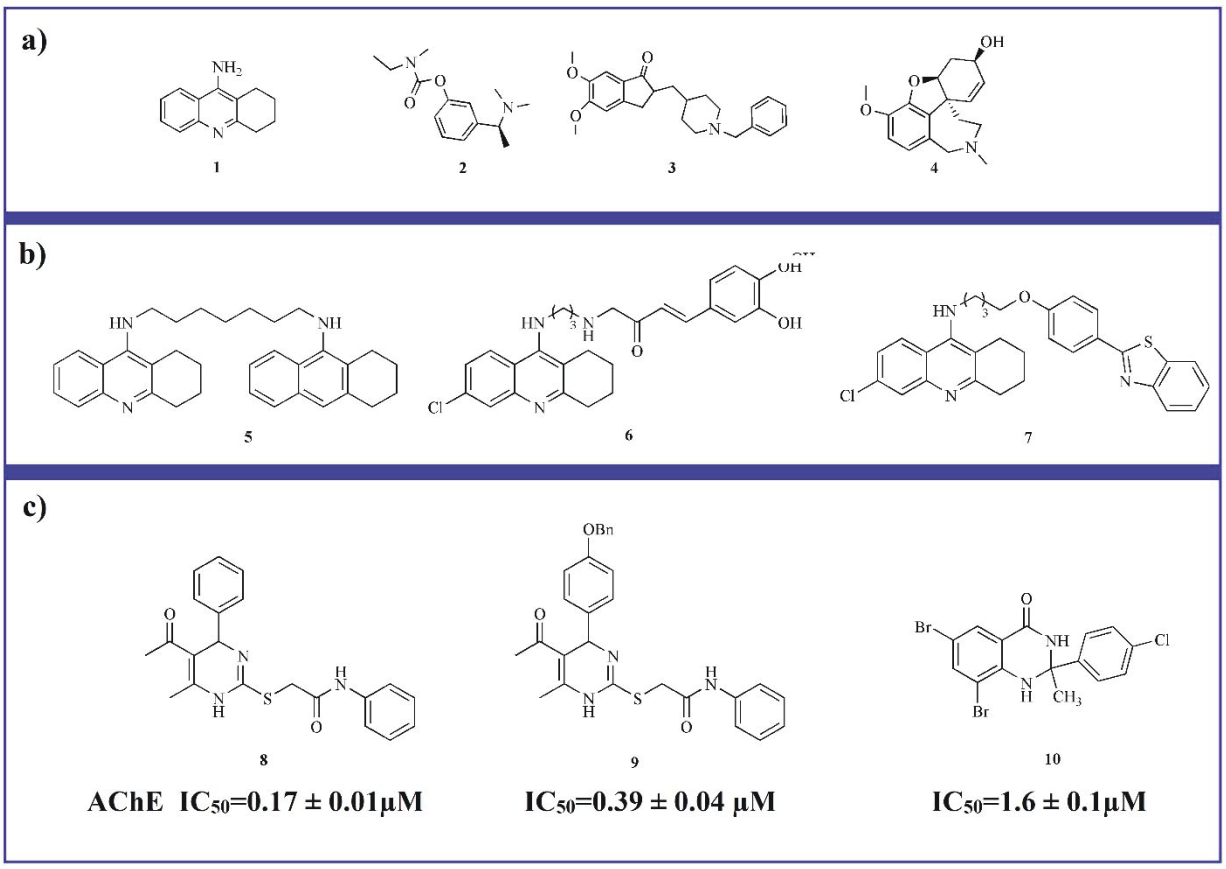



**Figure 1: a)** Chemical structure of approved drugs used for treatment of AD; **(b)** structures of some tacrine hybrids; bistacrine (**5**), tacrine-caffeic acid hybrid (**6**), tacrine-phenylbenzoheterocyclic hybrid (**7**)**; (c)** Structures of our previously reported of 2,3-dihydroquinazolin-4(*1H*)-one and dihydropyrimidines as cholinesterase inhibitor.

Quinazoline, a fused pyrimidine with a benzene ring **A** and a pyrimidine ring **B** (**Figure 2**), have a number of diversity points where structural modification can be made. In previous study, we have synthesized a number of 2,3-dihydroquinazolin-4(1H)-ones with diversity points on ring A. The variations at benzene ring resulted in good inhibition of cholinesterases (**Figure 2**). Pursuing the strategy of developing potent AChE inhibitors and to explore the diversity points on quinazoline core, we attempted to further optimize the N-1 position of 2,3-dihydro- quinazolin-4(*1H*)-one core. From our previous study, we have selected compounds containing un-

substituted phenyl ring fused with the pyrimidine ring having $IC_{50}$ values in the range of 9.9-80.3 μM. This modification was achieved by N-alkylation/benzylation of 2,3-dihydroquinazolin-4(*1H*)-one core using alkyl/benzyl halides in DCM.

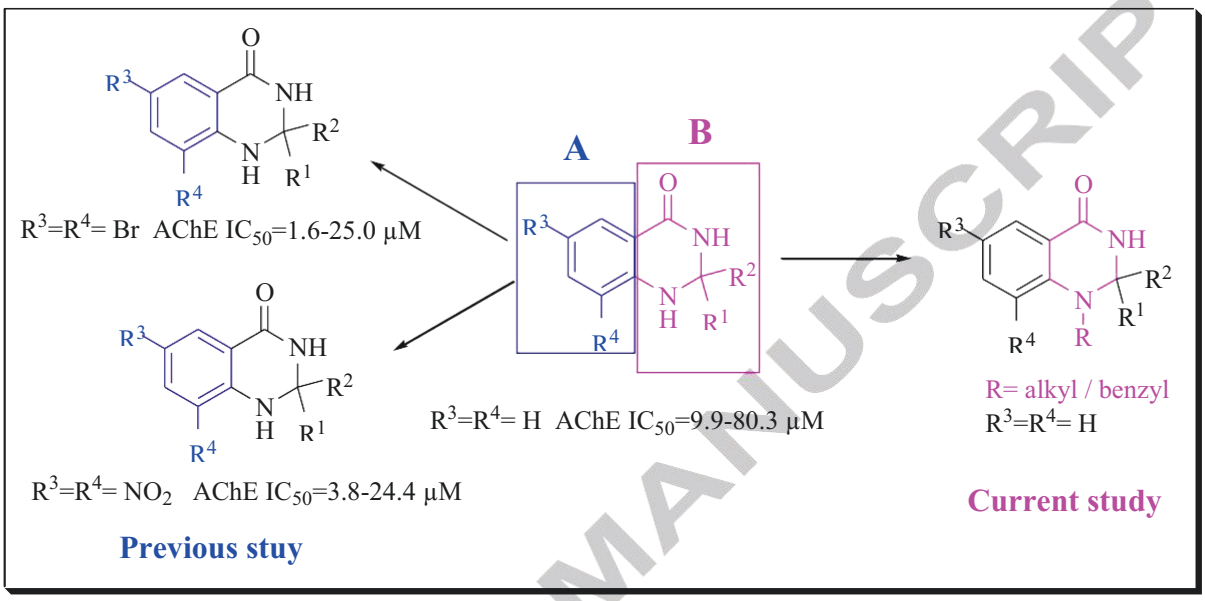


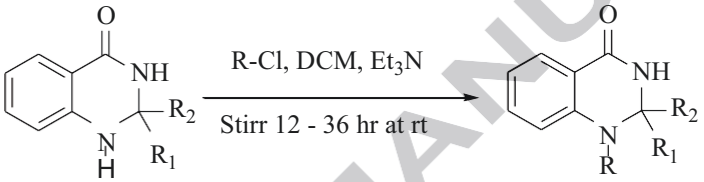

**Figure 2:** Design strategy for the synthesis of N-1 substituted quinazoline derivatives

## 2. Result and discussion

### 2.1. Chemistry

A number of diverse quinazoline derivatives have been synthesized and evaluated for various biological activities but studies on the synthesis of 2,2-disubstituted and 1,2,2-trisubstituted quinazoline moieties are very limited. Few researchers have attempted synthesis of 2,2-disubstituted quinazoline derivatives using various condensation and catalytic methodologies [37-40]. However, synthesis and biological activities study of 1,2-disubstituted and 1,2,2-trisubstituted quinazoline derivatives are scantly reported in literature. Quite a few reports are present in literature about the synthesis 1,2-disubstituted quinazoline derivatives by using N-substituted substrate like N-phenyl anthranilic acid or other analogues [41]. But to the best of our

knowledge, synthesis of 1,2,2-trisubstituted quinazoline derivatives have not been reported.. Instead of using *N*-substituted substrates (for example 2-(benzylamino)benzamide) for cyclization into quinazoline ring, we tried an alternative and simple method based on selective alkylation of N-1 unsubstituted quinalzoline core. Synthesis of 32 *N*-substituted quinazoline derivatives (**2a-af)** was accomplished by *N*-substitution of 2,3-dihydroquinazolin-4(1H)-one derivatives (**1a-h)** with alkyl/benzyl halides using triethylamine ($Et_3N$) as base and dichloromethane (DCM) as solvent under stirring conditions at room temperature (rt) **(Scheme 1).** Please see **Table 3** for chemical structures of synthesized quinazoline compounds (**2a-af**).




**1a-h** **2a-af**

**Scheme 1:** Synthesis of 1-alkyl/benzyl-2,2-disubstituted-2,3-dihydroquinazolin-4-one

Reaction time and yield of the synthesized derivatives **2a-af** varied depending upon the steric hindrance and reactivity starting material (**1a-h)**. Amongst all, highest yield (79%) with least reaction time (12 hrs) was obtained for compound **2a,** whereas, lowest yield (48%) with longest reaction time was reported for **2w.** Overall, reactivity of ethyl iodide toward *N*-substitution of compounds **1a-h** was higher as compared to other analogues of the same series as is evident from high yield (59-79%) and less reaction time (12-24 hrs). On the other hand, 2-chloro benzyl chloride was less reactive toward *N*-substitution of compounds **1a-h** due to highest reaction time (16-36 hrs) and less yield (48-71%) in comparison to other members of the respective series of compounds. Similarly, less yield and more reaction time was observed as $-CH_3$ group was

replaced with phenyl (**2e-h**, **2u-x**) or branched chain (**2y-af**) substituent at 2-position of **1a-h** which may be attributed to steric effect of bulky groups.

### 2.2. X-ray Crystallography Studies

Suitable colorless crystals of desired product **2a-af** were grown in ethyl acetate/ DCM and crystal analysis data was obtained for confirmation of the synthesized compounds. ORTEP diagram of representative compound **2j** is shown in **Figure 3a** whereas crystal data is presented in **Table 1.**

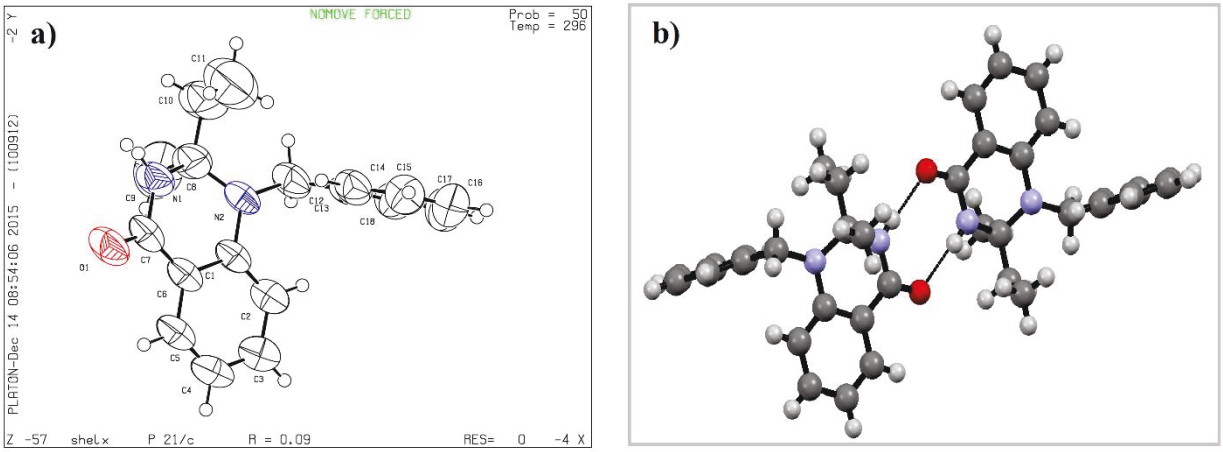



**Figure 3:** a) ORTEP diagram of compound**2j**; (b) Crystal packing diagram of compound **2j**

Selected bond lengths and bond angles of compounds **2j** and **2k** are presented in **Table 2** which shows that bond angles C1-N2-C8,C1-N2-C12 and N1-C8-N2 are higher in **2k** when compared to **2j** which may be due to steric hindrance of chloro group attached to 2-position of N-1 substituted benzyl group. Moreover crystal packing diagram of **2j (Figure 3b)** shows that only intermolecular hydrogen bonding exists in the molecules.

**Table 1:** XRD analysis data of compound **2j** (CCDC No. 1442212)

| **Compound** | | **2j** | |
|---|---|---|---|
| Chemical Formula | C18 H20 N2 O1 | | |
| *M* (g mol$^{-1}$) | 280.36 | | |
| Temperature(K) | 296(2) | | |
| crystal system | Monoclinic | | |
| space group | P 21/n | | |
| Cell volume | 1603.06 | | |
| a(Å) | 9.1245(18) | A | 90.0 |
| b (Å) | 18.508(4) | B | 115.544(5) |
| c (Å) | 10.5209(18) | Γ | 90.0 |
| O1 | -0.1514(4) | 0.43647(19) | 0.4936(3) |
| N1 | 0.0077(4) | 0.4300(2) | 0.3794(4) |
| H1 | 0.0588 | 0.4681 | 0.4228 |
| N2 | 0.0504(4) | 0.3202(2) | 0.2874(4) |
| C1 | -0.0838(5) | 0.2891(3) | 0.2984(4) |
| C2 | -0.1368(5) | 0.2201(3) | 0.2571(5) |
| H2 | -0.0850 | 0.1918 | 0.2157 |
| C3 | -0.2663(6) | 0.1917(3) | 0.2760(5) |
| H3 | -0.3017 | 0.1450 | 0.2458 |
| C4 | -0.3431(5) | 0.2321(3) | 0.3392(5) |
| H4 | -0.4290 | 0.2126 | 0.3531 |
| C5 | -0.2931(5) | 0.3000(3) | 0.3808(5) |
| H5 | -0.3457 | 0.3273 | 0.4230 |
| C6 | -0.1637(4) | 0.3304(2) | 0.3619(4) |
| C7 | -0.1062(5) | 0.4024(3) | 0.4147(5) |
| C8 | 0.0499(6) | 0.3988(3) | 0.2711(6) |
| C9 | -0.0862(10) | 0.4227(4) | 0.1180(7) |
| H9A | -0.0655 | 0.3990 | 0.0461 |
| H9B | -0.0822 | 0.4741 | 0.1077 |
| H9C | 0.1917 | 0.4091 | 0.1090 |
| C10 | 0.2076(11) | 0.4301(4) | 0.2914(11) |
| H10A | 0.2306 | 0.4134 | 0.2145 |
| H10B | 0.1941 | 0.4821 | 0.2819 |
| C11 | 0.3346(12) | 0.4174(5) | 0.4073(12) |
| H11A | 0.4276 | 0.4398 | 0.4038 |
| H11B | 0.3520 | 0.3662 | 0.4191 |
| H11C | 0.3187 | 0.4366 | 0.4851 |
| C12 | 0.1242(5) | 0.2768(3) | 0.2120(5) |
| H12A | 0.1948 | 0.3077 | 0.1884 |
| H12B | 0.0387 | 0.2594 | 0.1245 |
| C13 | 0.2204(5) | 0.2136(2) | 0.2952(5) |
| C14 | 0.3019(5) | 0.2149(3) | 0.4420(5) |
| H14 | 0.2936 | 0.2557 | 0.4902 |
| C15 | 0.3942(6) | 0.1575(3) | 0.5169(6) |
| H15 | 0.4486 | 0.1589 | 0.6146 |
| C16 | 0.4037(8) | 0.0980(3) | 0.4430(10) |
| H16 | 0.4661 | 0.0586 | 0.4915 |
| C17 | 0.3215(9) | 0.0955(4) | 0.2969(10) |
| H17 | 0.3270 | 0.0544 | 0.2482 |
| C18 | 0.2331(6) | 0.1536(3) | 0.2261(6) |
| H18 | 0.1802 | 0.1523 | 0.1282 |

**Table 2:** Selected bond angles and bond lengths of compound **2j** and **2k**

| Angle | 2j | 2k | Bonds | 2j | 2k |
|---|---|---|---|---|---|
| C1-N2-C8 | 117.3(4) | 120.5(3) | N1-C7 | 1.346(7) | 1.333(5) |
| C1-N2-C12 | 115.9(4) | 119.4(3) | N1-C8 | 1.469(8) | 1.473(6) |
| C8-N2-C12 | 117.1(4) | 116.5(3) | N2-C1 | 1.402(7) | 1.386(5) |
| N2-C1-C2 | 124.0(4) | 122.6(3) | N2-C8 | 1.465(7) | 1.455(6) |
| N1-C8-N2 | 106.8(4) | 109.2(4) | N2-C12 | 1.479(7) | 1.460(5) |

### 2.3. *In vitro* AChE and BChE inhibition assay

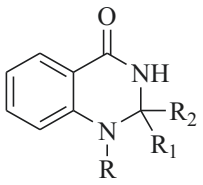
All the synthesized 32 compounds were evaluated for their AChE and BChE inhibition with donepezil and galatamine as reference drugs. The results are expressed as $IC_{50}$ and SI (selectivity index, given as $IC_{50}$ BChE/ $IC_{50}$ AChE and are tabulated in **Table 3**. Indeed, *N*-alkylation of the series of the compounds reported previously (*N*-unsubstituted) resulted in improved activity. All the compounds showed inhibition of both enzymes in the micromolar to submicromolar range. In general, improvement in AChE inhibition was observed with benzyl group at R. N-alkylation of the compounds already have alkyl group at $R^1$ and $R^2$ (**2a, 2i, 2m, 2q, 2y** and **2ac**) showed poor inhibition. However, benzyl substituent at N-1 was well tolerated and the compounds showed moderate inhibition. Among *N*-benzyl substituted derivatives, substitution of chloro group at ortho and para position contribute significantly to the biological activity.

Of the 32 derivatives, two compounds (**2ad** and **2af**) were found very active with their $IC_{50}$ values toward AChE in submicromolar range (0.8 μM and 0.6 μM respectively).These two compounds have 2,2-diisobutyl at $R_1$ and $R_2$. Furthermore, compounds having 4-Cl at benzyl group shows better activities and relocation of *p*-chloro group to *o*-position somewhat decrease

the ability of compound to inhibit AChE (4-chloro derivative **2af**, $IC_{50}$=0.06 μM*vs* 2-chloro derivative **2ae**, $IC_{50}$=13.2 μM). Compounds **2c, 2n, 2s, 2t** and **2z** show better activity than standard drug galatamine (**Table 3**). In our previous study, compound having methyl and 4-chlorophenyl group at R1 and R2 showed inhibition of 9.9μM [30]. *N*-alkylation of this compound resulted in significant increase in activity. Depending upon the alkylation (at R), except 2x, the resulted compounds **2u-x** has almost same or decreased potency. Compound **2x** showed good inhibition with $IC_{50}$=1.2 μM.

To evaluate the selectivity profile, $IC_{50}$ values of the synthesized compounds toward BChE were also determined. As seen from **Table 3**, compounds **2n, 2t, 2v, 2x, 2z, 2ad, 2af** shows better BChE inhibitory profile than donepezil. While, compounds **2h, 2v, 2x** and **2ae** shows better selectivity for BChE. Compound **2ae** exhibited excellent BChE inhibition with selectivity index of 2.6. Although the relocation of chloro group from *p*-position to *m*-position in **2ae** resulted in decrease in activity, however, it showed a balanced inhibition of both enzymes with selectivity index approaches to 0.8. Unfortunately, compound **2a, 2d, 2i, 2k, 2q, 2u**, and **2y** showed very poor BChE inhibition. Overall, the N-alkylation/benzylation have augmented the bioactivity of the synthesize compounds.

**Table 3:** *In vitro* AChE and BChE inhibitory activity of the synthesized compounds



**2a-af**

| Compound | $R^1$ | $R^2$ | R | $IC_{50}$ (μM ±SEM)[a] | | SI[b] |
|---|---|---|---|---|---|---|
| | | | | eeAChE | eqBChE | |
| **2a** | $CH_3$ | $CH_3$ | $CH_2CH_3$ | 33.8±1.32 | 70.9± 2.9 | 2.1 |
| **2b** | $CH_3$ | $CH_3$ | $C_6H_5CH_2$ | 6.4±0.16 | 22.4± 1.4 | 3.5 |
| **2c** | $CH_3$ | $CH_3$ | 2-Cl.$C_6H_5CH_2$ | 13.2± 1.80 | 50.2 ± 2.1 | 3.8 |
| **2d** | $CH_3$ | $CH_3$ | 4-Cl.$C_6H_5CH_2$ | **2.4 ± 0.52** | 16.5 ± 1.7 | 6.9 |
| **2e** | $CH_3$ | $C_6H_5$ | $CH_2CH_3$ | 18.6± 0.91 | 61.4 ± 2.6 | 3.3 |
| **2f** | $CH_3$ | $C_6H_5$ | $C_6H_5CH_2$ | 16.7± 1.03 | 21.7 ± 1.9 | 1.3 |
| **2g** | $CH_3$ | $C_6H_5$ | 2-Cl.$C_6H_5CH_2$ | 21.8± 1.20 | 19.6 ± 1.6 | 0.9 |
| **2h** | $CH_3$ | $C_6H_5$ | 4-Cl.$C_6H_5CH_2$ | 19.4± 0.31 | 20.5 ± 1.1 | 1.05 |
| **2i** | $CH_3$ | $CH_2CH_3$ | $CH_2CH_3$ | 21.7± 0.86 | 106.9 ±2.2 | 4.9 |
| **2j** | $CH_3$ | $CH_2CH_3$ | $C_6H_5CH_2$ | 11.8± 0.73 | 35.4 ± 1.3 | 3.0 |
| **2k** | $CH_3$ | $CH_2CH_3$ | 2-Cl.$C_6H_5CH_2$ | 13.0± 0.54 | 33.2 ± 2.8 | 2.5 |
| **2l** | $CH_3$ | $CH_2CH_3$ | 4-Cl.$C_6H_5CH_2$ | 7.3± 0.08 | 18.9 ± 2.1 | 2.6 |
| **2m** | $CH_2CH_3$ | $CH_2CH_3$ | $CH_2CH_3$ | 39.1± 0.60 | 107.2 ± 3.2 | 5.3 |
| **2n** | $CH_2CH_3$ | $CH_2CH_3$ | $C_6H_5CH_2$ | **1.8± 0.01** | **4.5**± 0.13 | 2.5 |
| **2o** | $CH_2CH_3$ | $CH_2CH_3$ | 2-Cl.$C_6H_5CH_2$ | 3.8± 0.01 | 11.02 ± 1.03 | 2.9 |
| **2p** | $CH_2CH_3$ | $CH_2CH_3$ | 4-Cl.$C_6H_5CH_2$ | 4.1± 0.03 | 23.7 ± 1.69 | 5.8 |
| **2q** | $CH_3$ | $CH_2CH_2CH_3$ | $CH_2CH_3$ | 32.3± 1.61 | 126.1 ± 4.08 | 3.9 |
| **2r** | $CH_3$ | $CH_2CH_2CH_3$ | $C_6H_5CH_2$ | 5.3± 0.13 | 44.5 ± 2.34 | 8.4 |
| **2s** | $CH_3$ | $CH_2CH_2CH_3$ | 2-Cl.$C_6H_5CH_2$ | 1.0 ± 0.01 | 6.5 ± 0.18 | 6.5 |
| **2t** | $CH_3$ | $CH_2CH_2CH_3$ | 4-Cl.$C_6H_5CH_2$ | 2.4± 0.43 | **4.6 ± 0.13** | 1.9 |
| **2u** | $CH_3$ | 4-Cl. $C_6H_4$ | $CH_2CH_3$ | 23.2± 1.12 | 97.4 ± 3.16 | 4.2 |
| **2v** | $CH_3$ | 4-Cl. $C_6H_4$ | $C_6H_5CH_2$ | 9.4± 0.88 | **3.76 ± 0.09** | 0.4 |
| **2w** | $CH_3$ | 4-Cl. $C_6H_4$ | 2-Cl.$C_6H_5CH_2$ | 19.3± 1.01 | 19.6 ± 1.29 | 1.0 |
| **2x** | $CH_3$ | 4-Cl. $C_6H_4$ | 4-Cl.$C_6H_5CH_2$ | **1.2 ± 0.09** | **4.48 ± 0.94** | 3.7 |
| **2y** | $CH_3$ | $CH_2CH(CH_3)_2$ | $CH_2CH_3$ | 43.2± 2.53 | 99.8 ± 3.21 | 2.3 |
| **2z** | $CH_3$ | $CH_2CH(CH_3)_2$ | $C_6H_5CH_2$ | **1.7 ± 0.02** | **3.57 ±0.08** | 2.1 |
| **2aa** | $CH_3$ | $CH_2CH(CH_3)_2$ | 2-Cl.$C_6H_5CH_2$ | 22.9± 1.30 | 46.0 ± 2.39 | 2.0 |
| **2ab** | $CH_3$ | $CH_2CH(CH_3)_2$ | 4-Cl.$C_6H_5CH_2$ | 9.2± 0.10 | 11.5 ± 0.12 | 1.2 |
| **2ac** | $CH_2CH(CH_3)_2$ | $CH_2CH(CH_3)_2$ | $CH_2CH_3$ | 16.1± 0.50 | 41.8 ± 1.88 | 2.6 |
| **2ad** | $CH_2CH(CH_3)_2$ | $CH_2CH(CH_3)_2$ | $C_6H_5CH_2$ | **0.8 ± 0.01** | **2.48 ± 0.01** | 3.1 |
| **2ae** | $CH_2CH(CH_3)_2$ | $CH_2CH(CH_3)_2$ | 2-Cl.$C_6H_5CH_2$ | 13.2± 0.80 | 10.56 ± 0.09 | 0.8 |
| **2af** | $CH_2CH(CH_3)_2$ | $CH_2CH(CH_3)_2$ | 4-Cl.$C_6H_5CH_2$ | **0.6± 0.01** | **1.56** ±0.08 | 2.6 |
| Donepezil | | | | 0.03 ± 0.01 | 5.4 ± 0.27 | 180 |
| Galantamine | | | | 4.0 ± 0.10 | 15.0 ± 0.67 | 3.7 |

[a] Values are expressed as mean of at least three experiments.

[b] Selectivity Index = $IC_{50}$ of BChE/ $IC_{50}$ of AChE. [c]Bold values are for highly active compounds than standard drug galatamine.

### 2.4. Docking study

Docking simulations were performed on the synthesized compounds with the active site of *Torpedo californica* (*Tc*AChE) by using GOLD (Genetic Optimization for Ligand Docking) suit v5.4.1 [42]. In previous study we also perform docking studies on human (hAChE). We have got the same results in current study and almost same binding modes were observed. The X-ray crystallographic structure of *Tc*AChE (PDB Code 1EVE) and *h*AChE (PDB Code 4EY7) in complex with donepezil were used as enzyme structures. Overall, *N*-benzylation of 2,3-dihydroquinazolin-4(1H)-one core resulted in increase in hydrophobic area of the compounds and strong binding than the unsubstituted compounds. In general, most of the compounds are embedded in bottom of the active site gorge and showed interactions with anionic substrate binding side, esteratic site and oxyanion hole residues. The orientation of the top-scored poses of the compounds with alkyl (methyl, ethyl or isobutyl) chains at $R^1$/R2 and N-1 (e.g. compound **2ac**) formed interactions with the amino acid residues located at the entry of the gorge (Asp72, Tyr121 or Tyr334). Introduction of benzyl group at N-1 position of these compounds resulted in the enhanced activity due to their extended interactions with amino acid residues at the bottom of the gorge (e.g. 20 fold increase in inhibition from **2ac** to **2af**). In **Figure 4a**, compound **2af** is colored by hydrophobic surfaces. The Gold Score of the most active compound **2af** is 72.7631. Indole ring of Trp84, an important residue of anionic site, is involved in the π-π stacking interaction with phenyl ring of quinazoline core and also with the benzyl ring. Substitution of halogen ring is considered as important in drug designing due to their effect in physico-chemical properties. Halogen-π interactions, a type of non-canonical interactions are well documented especially with aromatic ring of Tyr [43]. We have found a Cl-π interaction between 4-chloro at

benzyl ring and π-system of Tyr130. Interestingly, another interaction of Cl was observed with alkyl chain of Leu127. Another contribution of non-canonical interactions were observed between PAS residue Tyr121 and CH of isobutyl group. In fact, di-isobutyl is involved in many CH-π interaction with His440, Phe330, Phe331 and Tyr334 (**Figure 4b**). We can conclude here that the high activity of Cl containing compounds (especially at 4-position) is attributed to these interactions. A comparatively good activity was observe for compound **2x** (1.2 ± 0.09 μM) with 4-chlorophenyl/$CH_3$ group at 2,2- position of pyrimidine ring and 4-chlorobenzyl group at N-1. With a GoldScore of 68.7424, the molecule is oriented itself in a direction to form π-π stacking interaction with Trp84 and Tyr334. Tyr121 is involved in the conventional hydrogen bonding with N-3 of pyrimidine ring. A π-anionic interaction was also observed between Asp72 and phenyl ring of quinazoline core (**Figure 4c**).

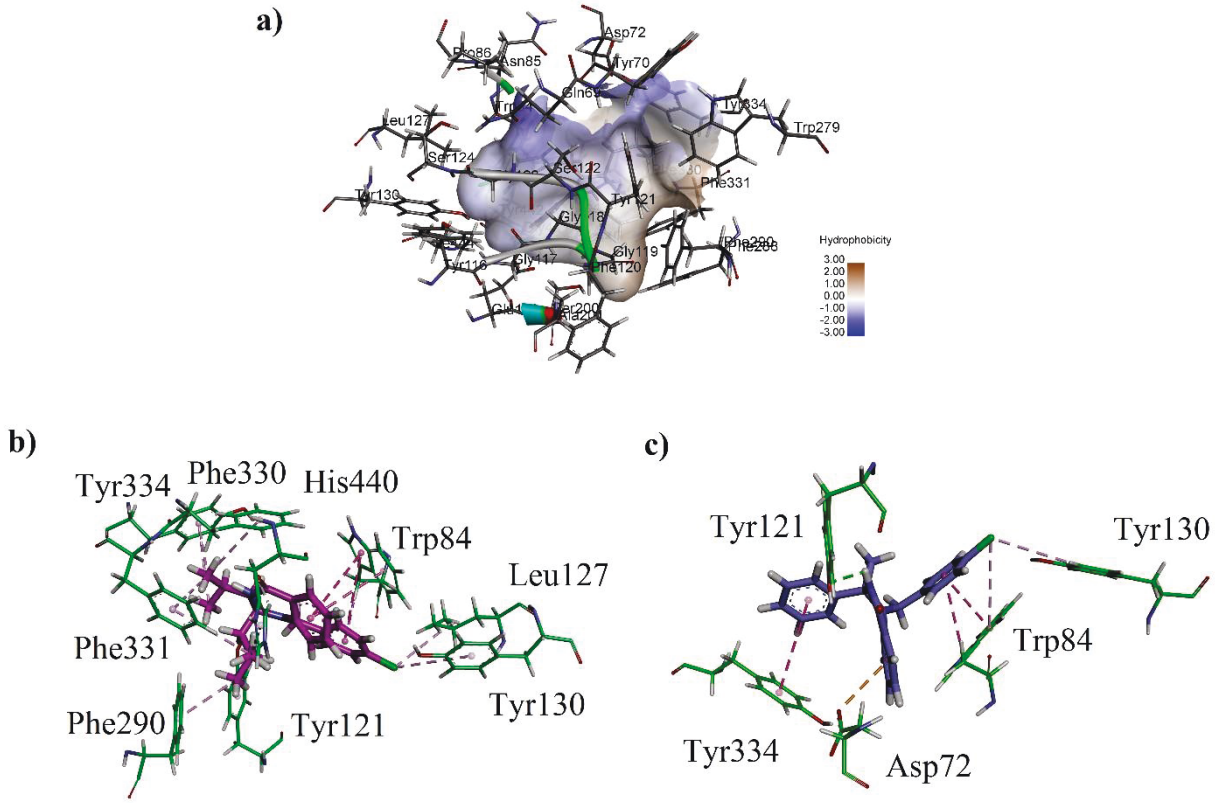



**Figure 4:** (**a**) Colored representation of compound **2af** by its hydrophobic surface; (**b**) Close-up depiction of the docking pose of most active compound **2af** in the binding site of 1EVE; (**c**) Close-up depiction of the docking pose of compound **2x** in the binding site of 1EVE. The key residues are represented as stick model.

Compound **2a** (23.8 ± 1.32 μM) with alkyl chains at N-1 and C-2 positions exhibited GoldScore 46.8669. It shows π-π stacking interaction with Trp84 and His440. A bifurcated conventional hydrogen bonding between Gly118 and Gly119 was also observed (**Figure 5a**). By the replacement of alkyl chain at N-1 with benzyl group **(2b**) the inhibition also increases. The computed GoldScore for the **2b** is 59.0461.Compound 2b forms two π-π stacking interaction with Trp84 and Tyr334. An ion-dipole interaction was established between NH of the ring and deprotonated Glu199. (**Figure 5b**). Another hydrogen bond interaction was observe between C=O and hydroxyl proton of Tyr130. Compound **2y** with $IC_{50}$=43.2± 2.53 μM showed poor

AChE inhibition. We have studied the binding mode of **2y** and compared its mode of orientation and interactions with the active compounds. As seen from **Figure 5c**, compound **2y** showed π-CH type weak interactions with Trp84, Phe330, Phe331 and Tyr334. An amide-π stacked interaction between Asn85 (located at the inner face of anionic site near Trp84) and ring A of quinazoline core.

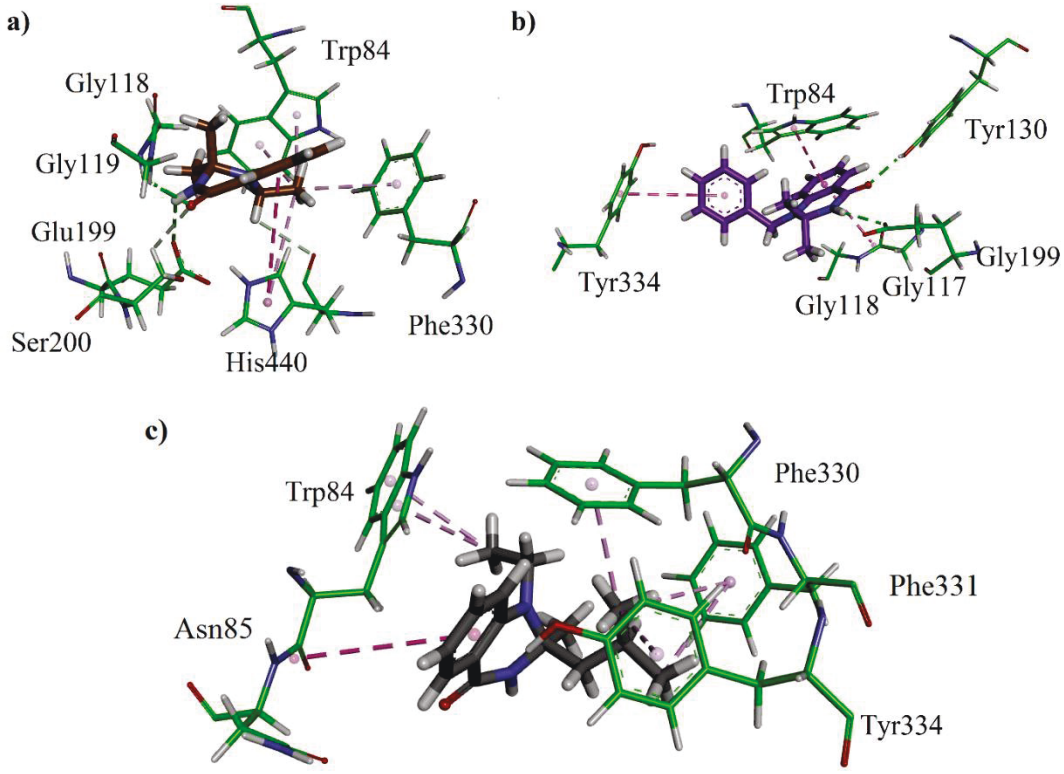



**Figure 5:** Close-up depiction of the docking pose of compounds (a) **2a** (b) **2b** (c) **2y** in the binding site of hBChE.

inspection of the top-scoring docking pose for **2af** (1.56 ± 0.08 μM) revealed that it is well stabilized in the active site of BChE and forms a number of hydrogen bond interactions with the key amino acids (**Figure 6a-b**). Gly116 and Gly117 located in oxyanion hole forms hydrogen bonds with carbonyl oxygen of quinazoline core. Tyr332 of PAS is involved in the π-π stacked

We have also performed the docking studies of the compounds targeting BChE. Visual

interactions with phenyl group at N-1 position. Leu286 and Val288 of acyl binding pocket forms π-alkyl interactions with the isobutyl groups at C-2 position of the quinazoline core. Phenyl ring of quinazoline core establishes π-π stacked interactions with choline binding site residue Trp82 and His438 of catalytic triad.

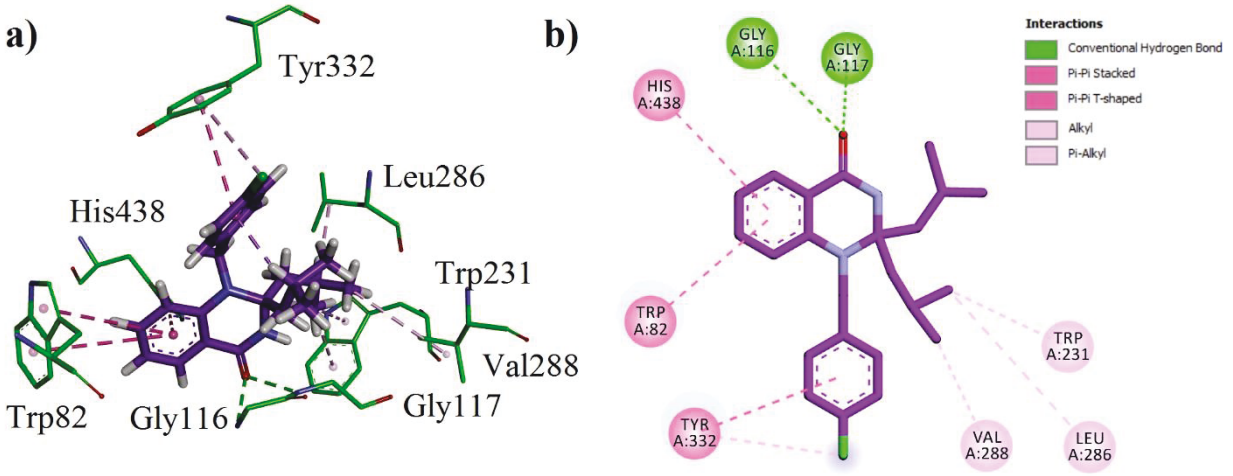



**Figure 6:** Close-up depiction of the docking pose of most active compound **2af** in the binding site of BChE (1P0I) (a) 3D pose; (b) 2D interactions.

### 2.5. Preliminary *in silico* pharmacokinetic

The purpose of this study is to predict the absorption of compounds in human intestine, their penetration across blood brain barrier (BBB) and their toxicities. *In-silico* pharmacokinetic studies of all synthesized compounds were carried out using online ADMET SAR server. These properties were compared with the standard drug donepezil (**Table S-1** in Supporting Information). All the molecules are predicted to be absorbed in human intestine (HIA) and cross BBB with almost similar probability. AMES toxicity and carcinogenic profile of all the

the BBB. **Figure 4** showed the comparative properties of some active compounds with standard drug donepezil. It can be seen from **Figure 7** that all the compounds can penetrate across the

synthesized compound showed that all the compounds are non-AMES toxic and non-carcinogens.

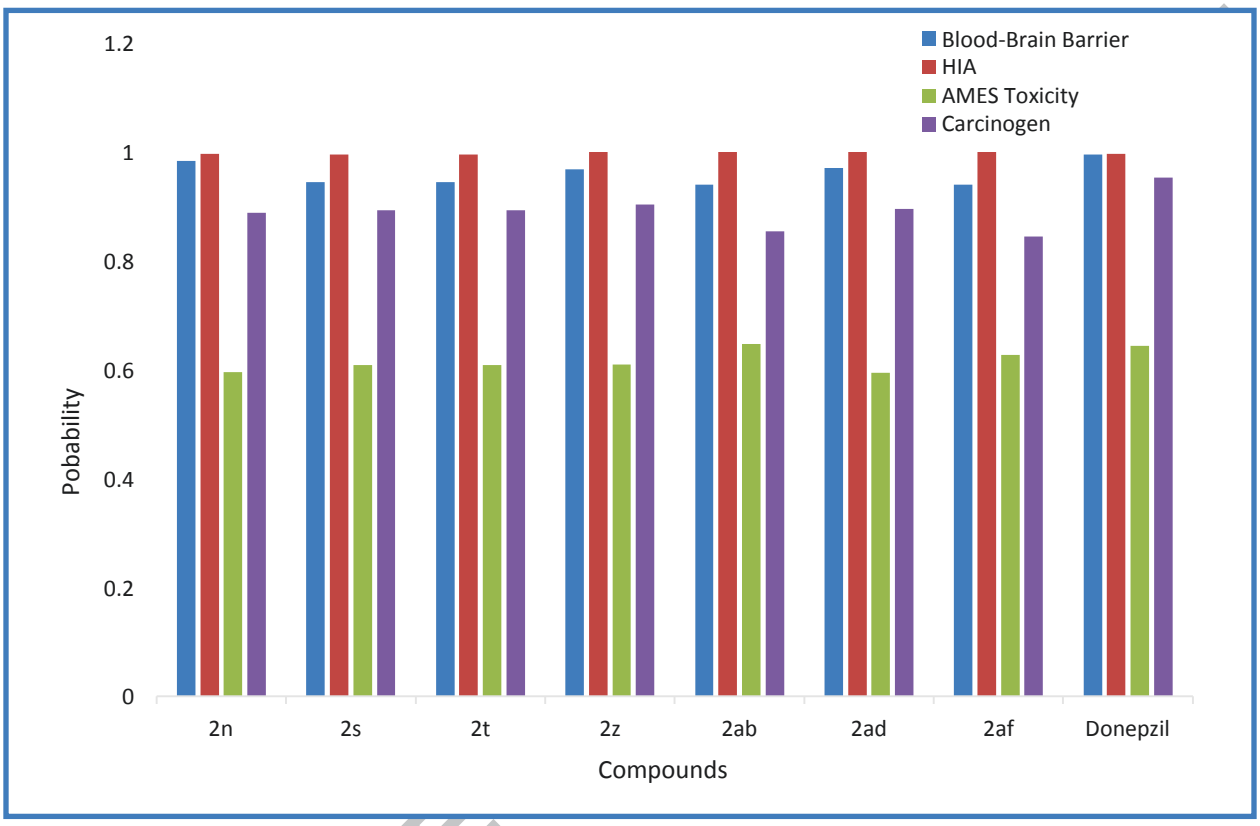



**Figure 7:** Comparative ADMET properties of the some of compounds with good AChE inhibition and standard drug donepezil predicted from admetSAR server.

### 3. Conclusion

In present study, N1-alkylation/benzylation of the 2,3-dihydroquinazolin-4(1*H*)-one core was carried out. All the 32 synthesized compounds were characterized by the spectroscopic technique and by single crystal X-ray diffraction studies. These compounds were evaluated for their inhibitory potential against AChE and BChE. Excellent activity of the N1-substituted quinazolines was observed. Two of them showed inhibitory activity in micromolar to

submicromolar range. Computational docking and preliminary pharmacokinetic studies were also carried out.

## 4. Materials and Methods

Analytical grade chemicals and reagents were used as received from commercial suppliers whereas further purification of organic solvents like methanol, ethyl acetate, dichloromethane (DCM) and n-hexane was done by using standard distillation conditions. For measurement of melting point, SMP30 Stuart Scientific melting point apparatus was used. Elemental analysis was carried out by using Elemental Vario EI III CHN analyzer and $^{1}$H and $^{13}$C NMR spectra were recorded on Bruker DRX 400 MHz NMR spectrometer at University of Science & Technology, Hefei, China. $^{1}$H and $^{13}$C chemical shifts are reported Vs $SiMe_4$ and were determined by reference to the residual $^{1}$H and $^{13}$C solvent peaks and coupling constants (*J*) were reported in Hz. Single beam X-rays crystallographic analysis was carried out by using Bruker KAPPA Apex II diffractometer at University of Sargodha, Pakistan.

### 4.1. General procedure for the synthesis of 1-alkyl/benzyl-2,2-disubstituted-2,3-dihydroquinazolin-one(6a-6af)

To a stirred solution of 2,2-disubstituted quinazolin-4(1*H*)-one **1a-h** (10 mmol) and $Et_3N$ (30 mmol) in DCM (10-20 ml), alkyl / benzyl halide (20 mmol) was added drop wise and stirring was continued for further 12-36 hrs at room temperature under nitrogen environment. After completion of the reaction, acidified water (5% HCl) was added to the reaction mixture and crude product was extracted with DCM. Evaporation of organic layer and subsequent

recrystallization with ethyl acetate resulted 1-alkyl/benzyl-2,2-disubstituted-2,3-dihydroquinazolin-4(1*H*)-one as desired product. Further purification of the product was accomplished by column chromatography using ethyl acetate and n-hexane (2:1) solvents and finally product was characterized by using X-rays crystallographic, elemental analysis and NMR techniques.

### *4.1.1. Synthesis of 1-ethyl-2,2-dimethyl-2,3-dihydro-1H-quinazolin-4-one (2a)*

Compound **2a** was synthesized by stirring **1a** (1.76 g, 10 mmol) and ethyl iodide (1.6 ml, 20 mmol) for 12 hrs adopting above mentioned general procedure as white solid. Yield 79%; m.p. 168-169 $^{0}$C; $^{1}$H NMR (400 MHz, CDCl3, 25 °C): δ = 7.88 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.38-7.34 (m, 1H, Ar*H*), 6.92 (s, 1H, N*H*), 6.84 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.79 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 3.57-3.51(m, 2H, $CH_2$), 1.18 (t, $^{3}J$ = 8 Hz, 3H, $CH_3$), 1.46 (s, 6H, 2×$CH_3$) ppm; $^{13}$C NMR (100.61 MHz, CDCl3, 25 °C): δ = 169.0, 147.0, 133.2, 129.2, 120.4, 119.6, 114.2, 66.8, 49.3, 31.9, 14.6; Anal. Calcd.for $C_{12}H_{16}N_2O$: C, 70.56; H, 7.90; N, 13.71. Found: C, 70.44; H, 7.96; N, 13.74.

### *4.1.2. Synthesis of 1-benzyl-2,2-dimethyl-2,3-dihydro-1H-quinazolin-4-one (2b)*

Above mentioned general procedure was adopted for the synthesis of **2b** by stirring **1a** (1.76 g, 10 mmol) and benzyl chloride (2.3 ml, 20 mmol) for 14 hrs as white solid; Yield 72%; m.p. 230-232 $^{0}$C; $^{1}$H NMR (400MHz, $CDCl_3$, 25 °C): δ = 7.91 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.36-7.33 (m, 1H, Ar*H*), 7.29-7.24 (m, 2H, Ar*H*), 7.18-7.14 (m, 1H, Ar*H*), 7.11 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 2H, Ar*H*), 6.91 (s, 1H, N*H*), 6.87 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.79 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 4.67 (s, 2H, $CH_2$), 1.46 (s, 6H, 2×$CH_3$) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 169.0, 147.0,

141.5, 133.2, 130.1, 129.2, 129.0, 127.7, 120.4, 119.6, 114.2, 66.8, 61.3, 31.9; Anal. Calcd.for $C_{17}H_{18}N_2O$: C, 76.66; H, 6.81; N, 10.52. Found: C, 76.74; H, 6.78; N, 10.49.

#### *4.1.3. Synthesis of 1-(2-chloro-benzyl)-2,2-dimethyl-2,3-dihydro-1H-quinazolin-4-one (2c)*

Compound **2c** was synthesized by utilizing general procedure by stirring **1a** (1.76 g, 10 mmol) and 2-chloro benzyl chloride (2.5 ml, 20 mmol) for 16 hrs as white solid; Yield 71%; m.p. 258-260 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.92 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.36-7.34 (m, 1H, Ar*H*), 7.25 (d, $^3J$= 7Hz, 1H, Ar*H*), 7.16-7.12 (m, 2H, Ar*H*), 7.09 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 6.89 (s, 1H, N*H*), 6.87 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.79 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 4.66 (s, 2H, $CH_2$), 1.49 (s, 6H, 2×$CH_3$) ppm; Anal. Calcd.for $C_{17}H_{17}ClN_2O$: C, 67.88; H, 5.7; N, 9.31. Found: C, 67.94; H, 5.68; N, 9.27.

#### *4.1.4. Synthesis of 1-(4-chloro-benzyl)-2,2-dimethyl-2,3-dihydro-1H-quinazolin-4-one (2d)*

Synthesis of compound **2d** was done by stirring **1a** (1.76 g, 10 mmol) and 4-chloro benzyl chloride (2.5 ml, 20 mmol) for 14 hrs following general procedure as white solid; Yield 72%; m.p. 256-258 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.92 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.37-7.34 (m, 1H, Ar*H*), 7.26 (d, $^3J$ = 8 Hz, 2H, Ar*H*), 7.11 (d, $^3J$ = 8 Hz, 2H, Ar*H* ), 6.91 (s, 1H, N*H*), 6.87 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.74 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 4.69 (s, 2H, $CH_2$), 1.49 (s, 6H, 2×$CH_3$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 169.2, 147.0, 140.1, 133.2, 131.9, 131.1, 129.9, 129.2, 120.1, 119.7, 114.2, 66.4, 61.1, 32.4; Anal. Calcd.for $C_{17}H_{17}ClN_2O$: C, 67.88; H, 5.7; N, 9.31. Found: C, 67.99; H, 5.71; N, 9.26.

#### *4.1.5. Synthesis of 1-ethyl-2-methyl-2-phenyl-2,3-dihydro-1H-quinazolin-4-one(2e)*

Compound **2e** was synthesized by stirring **1b** (2.38 g, 10 mmol) and ethyl iodide (1.6 ml, 20 mmol) for 16 hrs utilizing general procedure as white solid; Yield 71%; m.p. 230-232 $^{0}$C; $^{1}$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.90 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.38-7.34 (m, 1H, Ar*H*), 7.28-7.24 (m, 2H, Ar*H*), 7.16 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 2H, Ar*H*), 7.11-7.06 (m, 1H, Ar*H*), 6.88 (s, 1H, N*H*), 6.83 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.78 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 3.59-3.54 (m, 2H, $CH_2$), 1.88 (s, 3H, $CH_3$), 1.16 (t, $^{3}J$ = 8 Hz, 3H, $CH_3$) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 168.9, 147.1, 141.5, 133.2, 130.1, 129.4, 129.0, 127.7, 120.4, 119.4, 114.2, 71.4, 48.7, 29.1, 14.2; Anal. Calcd.for $C_{17}H_{18}N_2O$: C, 76.66; H, 6.81; N, 10.52. Found: C, 76.62; H, 6.85; N, 10.56.

### *4.1.6. Synthesis of 1-benzyl-2-methyl-2-phenyl-2,3-dihydro-1H-quinazolin-4-one (2f)*

Compound **2f** was synthesized by stirring **1b** (2.38 g, 10 mmol) and benzyl chloride (2.3 ml, 20 mmol) for 30hrs adopting above mentioned general procedure as white solid; Yield 54%; m.p. 301-302 $^{0}$C; $^{1}$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.91 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.37-7.34 (m, 1H, Ar*H*), 7.32-7.28 (m, 2H, Ar*H*), 7.27-7.23 (m, 2H, Ar*H*), 7.16 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 2H, Ar*H*), 7.11-7.06 (m, 4H, Ar*H*), 6.89 (s, 1H, N*H*), 6.85 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.76 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 4.69 (s, 2H, $CH_2$), 1.87 (s, 3H, $CH_3$) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 169.1, 146.8, 141.5, 133.2, 130.1, 129.4, 129.0, 127.7, 120.4, 119.2, 114.1, 71.3, 62.9, 29.1; Anal. Calcd.for $C_{22}H_{20}N_2O$: C, 80.46; H, 6.14; N, 8.53. Found: C, 80.62; H, 6.08; N, 8.46.

### *4.1.7. Synthesis of 1-(2-chloro-benzyl)-2-methyl-2-phenyl-2,3-dihydro-1H-quinazolin-4-one (2g)*

Synthesis of compound **2g** was achieved by stirring **1b** (2.38 g, 10 mmol) and 2-chloro benzyl chloride (2.5 ml, 20 mmol) for 36 hrs adopting above mentioned general procedure as white solid; Yield 51%; m.p. 315-316 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.88 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.38-7.34 (m, 1H, Ar*H*), 7.32-7.29 (m, 2H, Ar*H*), 7.22 (d, $^3J$ = 7 Hz,1H, Ar*H*), 7.16 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 2H, Ar*H*), 7.14-7.10 (m, 1H, Ar*H*), 7.07-7.04 (m, 3H, Ar*H*)), 6.93 (s, 1H, N*H*), 6.84 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.77 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 4.68 (s, 2H, $CH_2$), 1.89 (s, 3H, $CH_3$) ppm; Anal. Calcd. for $C_{22}H_{19}ClN_2O$: C, 72.82; H, 5.28; N, 7.72. Found: C, 72.69; H, 5.36; N, 7.85.

### *4.1.8. Synthesis of 1-(4-chloro-benzyl)-2-methyl-2-phenyl-2,3-dihydro-1H-quinazolin-4-one (2h)*

Above mentioned general procedure was utilized for the synthesis of **2h** by stirring **1b** (2.38 g, 10 mmol) and 4-chloro benzyl chloride (2.5 ml, 20 mmol) for 36 hrs as white solid; Yield 53%; m.p. 305-306 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.91 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.37-7.34 (m, 1H, Ar*H*), 7.31-7.27 (m, 2H, Ar*H*), 7.24 (d,$^3J$ = 8 Hz, 2H, Ar*H*), 7.17 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 2H, Ar*H*), 7.14-7.10 (m, 1H, Ar*H*), 7.08 (d, $^3J$ = 8 Hz, 2H, Ar*H*), 6.88 (s, 1H, N*H*), 6.84 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.77 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 4.68 (s, 2H, $CH_2$), 1.87 (s, 3H, $CH_3$) ppm; Anal. Calcd.for $C_{22}H_{19}ClN_2O$: C, 72.82; H, 5.28; N, 7.72. Found: C, 72.69; H, 5.36; N, 7.85.

### *4.1.9. Synthesis of 1,2-diethyl-2-methyl-2,3-dihydro-1H-quinazolin-4-one (2i)*

Compound **2i** was synthesized by stirring **1c** (1.9 g, 10 mmol) and ethyl iodide (1.6 ml, 20 mmol) for 12 hrs employing general procedure as white solid. Yield 77%; m.p. 169-170 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.90 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.38-7.34 (m, 1H, Ar*H*), 6.93 (s, 1H, N*H*), 6.87 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.76 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 3.59-3.53 (m, 2H, $CH_2$), 1.86-1.81 (m, 2H, $CH_2$), 1.64 (s, 3H, $CH_3$), 1.17 (t, $^3J$ =8 Hz, 3H, $CH_3$), 1.05 (t, $^3J$ =7 Hz, 3H, $CH_3$) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 168.8, 147.2, 133.2, 129.2, 120.4, 119.4, 114.2, 68.8, 47.8,35.8, 29.4, 14.6, 7.1; Anal. Calcd.for $C_{13}H_{18}N_2O$: C, 71.53; H, 8.31; N, 12.83. Found: C, 71.65; H, 8.24; N, 12.77.

***4.1.10. Synthesis of 1-benzyl-2-ethyl-2-methyl-2,3-dihydro-1H-quinazolin-4-one (2j)***

Synthesis of compound **2j** was achieved by stirring **1c** (1.9 g, 10 mmol) and benzyl chloride (2.3 ml, 20 mmol) for 12 h following general procedure as white solid; Yield 71%; m.p. 239-241 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.89 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.36-7.33 (m, 1H, Ar*H*), 7.28-7.23 (m, 2H, Ar*H*), 7.19-7.14 (m, 1H,Ar*H*), 7.13(dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 2H, Ar*H*), 6.91 (s, 1H, N*H*), 6.86 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.77 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 4.70 (s, 2H, $CH_2$), 1.86-1.82 (m, 2H, $CH_2$), 1.64 (s, 3H, $CH_3$), 1.04 (t, $^3J$ =7 Hz, 3H, $CH_3$) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 169.3, 147.4, 141.0, 133.2, 130.1, 129.2, 129.0, 127.7, 120.4, 119.5, 114.2, 69.8, 61.0, 39.4, 29.4, 7.3; Anal. Calcd.for $C_{18}H_{20}N_2O$: C, 77.11; H, 7.19; N, 9.99. Found: C, 77.03; H, 7.22; N, 10.04.

***4.1.11. Synthesis of 1-(2-chloro-benzyl)-2-ethyl-2-methyl-2,3-dihydro-1H-quinazolin-4-one (2k)***

Compound **2k** was synthesized by stirring **1c** (1.9 g, 10 mmol) and 2-chloro benzyl chloride (2.5 ml, 20 mmol) for 16 h following general procedure as white solid; Yield 66%; m.p. 274-276 $^0$C;

$^{1}$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.91 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.37-7.33 (m, 1H, Ar*H*), 7.28 (d, $^{3}J$ = 7 Hz, 1H, Ar*H*), 7.17-7.13 (m, 2H, Ar*H*), 7.10 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 6.91 (s, 1H, N*H*), 6.86 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.79 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 4.69 (s, 2H, $CH_2$), 1.90-1.86 (m, 2H, $CH_2$), 1.64 (s, 3H, $CH_3$), 1.05 (t, $^{3}J$ =7 Hz, 3H, $CH_3$) ppm; Anal. Calcd. for $C_{18}H_{19}ClN_2O$: C, 68.67; H, 6.08; N, 8.91. Found: C, 68.52; H, 6.12; N, 9.01.

### *4.1.12. Synthesis of 1-(4-chloro-benzyl)-2-ethyl-2-methyl-2,3-dihydro-1H-quinazolin-4-one (2l)*

Synthesis of compound **2l** was done by stirring **1c** (1.9 g, 10 mmol) and 4-chloro benzyl chloride (2.5ml, 20 mmol) for 14 hrs following general procedure as white solid; Yield 68%; m.p. 268-270 $^{0}$C; $^{1}$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.92 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.36-7.33 (m, 1H, Ar*H*), 7.22 (d, $^{3}J$ = 8 Hz , 2H, Ar*H*), 7.09 (d, $^{3}J$ = 8 Hz, 2H, Ar*H*), 6.89 (s, 1H, N*H*), 6.85 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.78 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 4.67 (s, 2H, $CH_2$), 1.89-1.86 (m, 2H, $CH_2$), 1.63 (s, 3H, $CH_3$), 1.03 (t, $^{3}J$ = 8 Hz, 3H, $CH_3$) ppm; Anal. Calcd.for $C_{18}H_{19}ClN_2O$: C, 68.67; H, 6.08; N, 8.91. Found: C, 68.59; H, 6.10; N, 8.98.

### *4.1.13. Synthesis of 1,2,2-triethyl-2,3-dihydro-1H-quinazolin-4-one (2m)*

Compound **2m** was synthesized by stirring **1d** (2.0 g, 10 mmol) and ethyl iodide (1.6 ml, 20 mmol) for 12 hrs following above mentioned general procedure as white solid; Yield 79%; m.p. 171-172 $^{0}$C; $^{1}$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.89 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.38-7.34 (m, 1H, Ar*H*), 6.91 (s, 1H, N*H*), 6.87 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.80 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 3.60-3.56 (m, 2H, $CH_2$), 1.90-1.85 (m, 4H, 2×$CH_2$), 1.18 (t, $^{3}J$ = 7 Hz, 3H, $CH_3$), 1.07 (t, $^{3}J$ =7 Hz, 6H, 2×$CH_3$) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 169.2, 146.9, 133.2, 129.1,

119.9, 119.2, 114.2, 70.9,45.2, 39.8, 14.3, 8.8; Anal. Calcd.for $C_{14}H_{20}N_2O$: C, 72.38; H, 8.68; N, 12.06. Found: C, 72.30; H, 8.72; N, 12.02.

#### *4.1.14. Synthesis of 1-benzyl-2,2-diethyl-2,3-dihydro-1H-quinazolin-4-one (2n)*

Above mentioned general procedure was used for the synthesis of **2n** by stirring **1d** (2.0 g, 10 mmol) and benzyl chloride (2.3 ml, 20 mmol) for 18 hrs as white solid; Yield 77%; m.p. 249-251 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.88 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.36-7.33 (m, 1H, Ar*H*), 7.29-7.25 (m, 2H, Ar*H*), 7.17-7.13 (m, 1H, Ar*H*), 7.15 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 2H, Ar*H*), 6.90 (s, 1H, N*H*), 6.86 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.80 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 4.69 (s, 2H, $CH_2$), 1.93-1.88 (m, 4H, 2×$CH_2$), 1.05 (t, $^3J$ =7 Hz, 6H, 2×$CH_3$) ppm; $^{13}$C NMR (100MHz, $CDCl_3$, 25 °C): δ = 169.8, 147.2, 141.5, 133.2, 130.1, 129.1, 129.0, 127.4, 119.9, 119.0, 114.2, 71.0, 60.9, 39.8, 9.2; Anal. Calcd.for $C_{19}H_{22}N_2O$: C, 77.52; H, 7.53; N, 9.52. Found: C, 77.63; H, 7.48; N, 9.51.

#### *4.1.15. Synthesis of 1-(2-chloro-benzyl)-2,2-diethyl-2,3-dihydro-1H-quinazolin-4-one (2o)*

Compound **2o** was obtained by stirring **1d** (2.0 g, 10 mmol) and 2-chlorobenzyl chloride (2.5 ml, 20 mmol) for 18 h following general procedure as white solid; Yield 63%; m.p. 289-291 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.89 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.37-7.33 (m, 1H, Ar*H*), 7.24 (d, $^3J$ = 7 Hz, 1H, Ar*H*), 7.16-7.11 (m, 2H, Ar*H*), 7.09 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 6.91 (s, 1H, N*H*), 6.87 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.79 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 4.68 (s, 2H, $CH_2$), 1.89-1.85 (m, 4H, 2×$CH_2$), 1.06 (t, $^3J$ =7 Hz, 6H, 2×$CH_3$); Anal. Calcd.for $C_{19}H_{21}ClN_2O$: C, 69.40; H, 6.44; N, 8.52. Found: C, 69.33; H, 6.47; N, 8.55.

#### *4.1.16.* Synthesis of 1-(4-chloro-benzyl)-2,2-diethyl-2,3-dihydro-1H-quinazolin-4-one (2p)

Similar synthetic procedure as described above was utilized to synthesize **2p** by reacting **1d** (2.0 g, 10 mmol) with 4-chloro benzyl chloride (2.5 ml, 20 mmol) under stirring conditions for 18 hrs. White solid; Yield 64%; m.p. 282-284 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.90 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.36-7.32 (m, 1H, Ar*H*), 7.23 (d, $^3J$= 8Hz, 2H, Ar*H*), 7.08 (d, $^3J$ = 8 Hz, 2H, Ar*H*), 6.94 (s, 1H, N*H*), 6.85 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.73 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 4.66 (s, 2H, $CH_2$), 1.92-1.87 (m, 4H, 2×$CH_2$), 1.05 (t, $^3J$ =7 Hz, 6H, 2×$CH_3$) ppm; Anal. Calcd.for $C_{19}H_{21}ClN_2O$: C, 69.40; H, 6.44; N, 8.52. Found: C, 69.44; H, 6.41; N, 8.49.

#### *4.1.17. Synthesis of 1-ethyl-2-methyl-2-propyl -2,3-dihydro-1H-quinazolin-4-one (2q)*

Compound **2q** was synthesized by stirring **1e** (2.0 g, 10 mmol) and ethyl iodide (1.6 ml, 20 mmol) for 12 hrs following above mentioned general procedure to afford white solid as desired product; Yield 72%; m.p. 173-172 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.90 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.38-7.34 (m, 1H, Ar*H*), 6.89 (s, 1H, N*H*), 6.86 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.77 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 3.60-3.55 (m, 2H, $CH_2$), 1.83-1.78 (m, 2H, $CH_2$), 1.59 (s, 3H, $CH_3$), 1.41-1.37 (m, 2H, $CH_2$), 1.15 (t, $^3J$ = 8 Hz, 3H, $CH_3$), 1.06 (t, $^3J$ =7 Hz, 3H, $CH_3$) ppm; $^{13}$C NMR (100 MHz, [d]$CDCl_3$, 25 °C): δ = 168.6, 146.7, 133.1, 128.6, 119.3, 118.6, 113.4, 66.8, 46.5, 44.6, 27.3, 15.8, 14.8, 14.0; Anal. Calcd.for $C_{14}H_{20}N_2O$: C, 72.38; H, 8.68; N, 12.06. Found: C, 72.46; H, 8.60; N, 12.02.

#### *4.1.18. Synthesis of 1-benzyl-2-methyl-2-propyl-2,3-dihydro-1H-quinazolin-4-one (2r)*

Above mentioned general procedure was adopted to achieve synthesis of **2r** by stirring **1e** (2.0 g, 10 mmol) and benzyl chloride (2.3 ml, 20 mmol) for 18 hrs. White solid; Yield 68%; m.p. 259-261 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.88 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.36-

7.32 (m, 1H, Ar*H*), 7.29-7.25 (m, 2H, Ar*H*), 7.15-7.11 (m, 1H, Ar*H*), 7.09 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 2H, Ar*H*), 6.92 (s, 1H, N*H*), 6.86 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.77 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 4.68 (s, 2H, $CH_2$), 1.86-1.81 (m, 2H, $CH_2$), 1.59 (s, 3H, $CH_3$), 1.41-1.37 (m, 2H, $CH_2$), 0.99 (t, $^{3}J$ =7 Hz, 3H, $CH_3$) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 169.2, 147.1, 140.8, 133.1, 129.8, 129.1, 128.6, 127.0, 119.3, 118.9, 113.4, 66.2, 59.8, 43.8, 27.9, 16.8, 14.3; Anal. Calcd.for $C_{19}H_{22}N_2O$: C, 77.52; H, 7.53; N, 9.52. Found: C, 77.66; H, 7.49; N, 9.45.

### *4.1.19. Synthesis of 1-(2-chloro-benzyl)-2-methyl-2-propyl-2,3-dihydro-1H-quinazolin-4-one (2s)*

Compound **2s** was synthesized by stirring **1e** (2.0 g, 10 mmol) and 2-chloro benzyl chloride (2.5 ml, 20 mmol) for 20 hrs following general procedure as white solid; Yield 57%, m.p. 298-300 $^{0}$C; $^{1}$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.92 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.36-7.32 (m, 1H, Ar*H*), 7.24 (d, $^{3}J$ = 7 Hz, 1H, Ar*H*), 7.12-7.08 (m, 2H, Ar*H*), 7.07 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 6.90 (s, 1H, N*H*), 6.84 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.73 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 4.66 (s, 2H, $CH_2$), 1.83-1.79 (m, 2H, $CH_2$), 1.58 (s, 3H, $CH_3$), 1.41-1.36 (m, 2H, $CH_2$), 1.02 (t, $^{3}J$ =7 Hz, 3H, $CH_3$) ppm; Anal. Calcd.for $C_{19}H_{21}ClN_2O$: C, 69.4; H, 6.44; N, 8.52. Found: C, 69.35; H, 6.51; N, 8.49.

### *4.1.20. Synthesis of 1-(4-chloro-benzyl)-2-methyl-2-propyl-2,3-dihydro-1H-quinazolin-4-one (2t)*

Same general procedure as described above was adopted for the synthesis of **2t** by stirring **1e** (2.0 g, 10 mmol) and 4-chloro benzyl chloride (2.5 ml, 20 mmol) for 16 hrs. White solid; Yield 61%; m.p. 291-293 $^{0}$C; $^{1}$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.90 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.37-7.33 (m, 1H, Ar*H*), 7.22 (d, $^{3}J$ = 8 Hz, 2H, Ar*H*), 7.07 (d, $^{3}J$ = 8 Hz, 2H, Ar*H*),

6.93 (s, 1H, N*H*), 6.86 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.78 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 4.67 (s, 2H, $CH_2$), 1.84-1.80 (m, 2H, $CH_2$), 1.60 (s, 3H, $CH_3$), 1.40-1.37 (m, 2H, $CH_2$), 0.99 (t, $^{3}J$ =7 Hz, 3H, $CH_3$) ppm; Anal. Calcd.for $C_{19}H_{21}ClN_2O$: C, 69.4; H, 6.44; N, 8.52. Found: C, 69.47; H, 6.41; N, 8.47.

***4.1.21. Synthesis of 2-(4-chloro-phenyl)-1-ethyl-2-methyl-2,3-dihydro-1H-quinazolin-4-one (2u)***

Compound **2u** was synthesized by exploiting general procedure by stirring a mixture of **1f** (2.7 g, 10 mmol) and ethyl iodide (1.6 ml, 20 mmol) for 16 hrs as white solid. Yield 68%; m.p. 269-268 °C; $^{1}$H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ = 7.89 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.37-7.33 (m, 1H, Ar*H*), 7.27 (d, $^{3}J$ = 8 Hz, 2H, Ar*H* ), 7.11 (d, $^{3}J$ = 8 Hz, 2H, Ar*H*), 6.90 (s, 1H, N*H*), 6.86 (t, $^{3}J$ = 8 Hz, 1H, Ar*H*), 6.80 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 3.56-3.52 (m, 2H, $CH_2$), 1.86 (s, 3H, $CH_3$), 1.16 (t, $^{3}J$ = 7 Hz, 3H, $CH_3$) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$,25 °C), δ=168.7, 145.9, 143.5, 134.3, 128.8, 128.7, 128.5, 126.8, 119.6, 115.1, 72.0, 45.2, 30.0, 15.2.

***4.1.22. Synthesis of 1-benzyl-2-(4-chloro-phenyl)-2-methyl-2,3-dihydro-1H-quinazolin-4-one (2v)***

Synthesis of compound **2v** was done by stirring a mixture of **1f** (2.7 g, 10 mmol) and benzyl chloride (2.3 ml, 20 mmol) for 32 hrs as white solid. Yield 56%; m.p. 348-349 °C; $^{1}$H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ = 7.87 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.37-7.33 (m, 1H, Ar*H*), 7.29 (d, $^{3}J$ = 8 Hz, 2H, Ar*H*), 7.26-7.21 (m, 2H, Ar*H*), 7.13-7.10 (m, 1H, Ar*H*), 7.08 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 2H, Ar*H*), 6.93 (s, 1H, N*H*), 6.86 (t, $^{3}J$ = 8 Hz, 1H, Ar*H*), 6.79 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 4.66 (s, 2H, $CH_2$), 1.88 (s, 3H, $CH_3$)ppm; Anal. Calcd. for $C_{22}H_{19}ClN_2O$: C, 72.82; H, 5.28; N, 7.72. Found: C, 73.01; H, 5.15; N, 7.75.

***4.1.23. Synthesis of 1-(2-chloro-benzyl)-2-(4-chloro-phenyl)-2-methyl-2,3-dihydro-1H-quinazolin-4-one (2w)***

Synthesis of compound **2w** was done by stirring a mixture of **1f** (2.7 g, 10 mmol) and 2-chloro benzyl chloride (2.5 ml, 20 mmol) for 36 hrs as white solid. Yield 48%; $^{1}$H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ = 7.86 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.38-7.34 (m, 1H, Ar*H*), 7.28 (d, $^{3}J$ = 8 Hz, 2H, Ar*H* ), 7.21 (d, $^{3}J$ = 7 Hz, 1H, Ar*H*), 7.13 (d, $^{3}J$ = 8 Hz, 2H, Ar*H*), 7.10-7.06 (m, 3H, Ar*H*), 6.93 (s, 1H, N*H*), 6.85 (t, $^{3}J$ = 8 Hz, 1H, Ar*H*), 6.81 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 4.66 (s, 2H, $CH_2$), 1.86 (s, 3H, $CH_3$) ppm; Anal. Calcd. for $C_{22}H_{18}Cl_2N_2O$: C, 66.51; H, 4.57; N, 7.05. Found: C, 66.41; H, 4.65; N, 7.15.

***4.1.24. Synthesis of 1-(4-chloro-benzyl)-2-(4-chloro-phenyl)-2-methyl-2,3-dihydro-1H-quinazolin-4-one (2x)***

Synthesis of compound **2x** was done by stirring a mixture of **1f** (2.7 g, 10 mmol) and 4-chloro benzyl chloride (2.5 ml, 20 mmol) for 36 hrs as white solid. Yield 52%; $^{1}$H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ = 7.87 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.37-7.34 (m, 1H, Ar*H*), 7.27 (d, $^{3}J$ = 8 Hz, 2H, Ar*H* ), 7.21 (d, $^{3}J$ = 8 Hz, 2H, Ar*H*), 7.10 (d, $^{3}J$ = 8 Hz, 2H, Ar*H*), 7.06 (d, $^{3}J$ = 8 Hz, 2H, Ar*H*), 6.92 (s, 1H, N*H*), 6.84 (t, $^{3}J$ = 8 Hz, 1H, Ar*H*), 6.80 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 4.66 (s, 2H, $CH_2$), 1.86 (s, 3H, $CH_3$) ppm; Anal. Calcd. for$C_{22}H_{18}Cl_2N_2O$: C, 66.51; H, 4.57; N, 7.05. Found: C, 66.38; H, 4.65; N, 7.15.

***4.1.25. Synthesis of 1-ethyl-2-isobutyl-2-methyl-2,3-dihydro-1H-quinazolin-4-one (2y)***

Compound **2y** was synthesized by stirring reaction mixture of **1g** (2.2 g, 10 mmol) and ethyl iodide (1.6 ml, 20 mmol) for 16 hrs following general procedure. White solid; Yield 69%; m.p. 174-173 $^{0}$C; $^{1}$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.89 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*),

7.38-7.34 (m, 1H, Ar*H*), 6.90 (s, 1H, N*H*), 6.84 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.76 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 3.56-351(m, 2H, $CH_2$), 1.91-1.87 (m, 1H, CH), 1.76 (d, $^{3}J$ = 7 Hz, 2H, $CH_2$), 1.58 (s, 3H, $CH_3$), 1.15 (t, $^{3}J$ = 8 Hz, 3H, $CH_3$), 1.07 (t, $^{3}J$ = 6 Hz, 6H, 2×$CH_3$) ppm; Anal. Calcd.for$C_{15}H_{22}N_2O$: C, 73.13; H, 9.00; N, 11.37. Found: C, 73.01; H, 9.06; N, 11.41.

***4.1.26. Synthesis of 1-benzyl-2-isobutyl-2-methyl-2,3-dihydro-1H-quinazolin-4-one (2z)***

Synthesis of compound **2z** was done by stirring reaction mixture of **1g** (2.2 g, 10 mmol) with benzyl chloride (2.3 ml, 20 mmol) for 24 hrs following general procedure as white solid; Yield 61%; m.p. 238-240 $^{0}$C; $^{1}$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.90 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.36-7.32 (m, 1H, Ar*H*), 7.26-7.22 (m, 2H, Ar*H*), 7.15-7.11 (m, 1H, Ar*H*), 7.10 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 2H, Ar*H*), 6.91 (s, 1H, N*H*), 6.86 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.78 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 4.67 (s, 2H, $CH_2$), 1.89-1.85 (m, 1H, CH), 1.75 (d, $^{3}J$ = 7 Hz, 2H, $CH_2$), 1.58 (s, 3H, $CH_3$), 1.05 (t, $^{3}J$ = 6 Hz, 6H, 2×$CH_3$) ppm; $^{13}$C NMR (100 MHz, [d]$CDCl_3$, 25 °C): δ = 167.8, 147.1, 140.8, 133.1, 129.7, 129.0, 128.6, 127.0, 119.1, 118.6, 113.4, 66.1, 58.6, 50.8, 27.3, 23.8, 19.3; Anal. Calcd.for $C_{20}H_{24}N_2O$: C, 77.89; H, 7.84; N, 9.08. Found: C, 77.96; H, 7.79; N, 9.02.

***4.1.27. Synthesis of 1-(2-chloro-benzyl)-2-isobutyl-2-methyl-2,3-dihydro-1H-quinazolin-4-one (2aa)***

Above mentioned general procedure was adopted for the synthesis of **2aa** by stirring **1g** (2.2 g, 10 mmol) with 2-chloro benzyl chloride (2.5 ml, 20 mmol) for 36 hrs. White solid; Yield 53%; m.p. 299-301 $^{0}$C; $^{1}$H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ = 7.90 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 7.36-7.32 (m, 1H, Ar*H*), 7.26 (d, $^{3}J$ = 7 Hz, 1H, Ar*H*), 7.13-7.09 (m, 2H, Ar*H*), 7.06 (dd, $^{3}J$ = 8 Hz, $^{4}J$ = 1 Hz, 1H, Ar*H*), 6.90 (s, 1H, N*H*), 6.86 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.79 (d, $^{3}J$ = 8

Hz, 1H, Ar*H*), 4.68 (s, 2H, $CH_2$), 1.90-1.85 (m, 1H, CH), 1.76 (d, $^3J$ = 7 Hz, 2H, $CH_2$), 1.58 (s, 3H, $CH_3$), 1.05 (t, $^3J$ = 6 Hz, 6H, 2×$CH_3$) ppm; Anal. Calcd.for $C_{20}H_{23}ClN_2O$: C, 70.06; H, 6.76; N, 8.17. Found: C, 69.89; H, 6.79; N, 8.21.

### *4.1.28. Synthesis of 1-(4-chloro-benzyl)-2-isobutyl-2-methyl-2,3-dihydro-1H-quinazolin-4-one (2ab)*

Synthesis of compound **2ab** was achieved by stirring **1g** (2.2 g, 10 mmol) with 4-chloro benzyl chloride (2.5 ml, 20 mmol) for 30 hrs following general procedure. White solid; Yield 56%; m.p. 286-288 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.91 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.35-7.32 (m, 1H, Ar*H*), 7.23 (d, $^3J$ = 8 Hz, 2H, Ar*H*), 7.08 (d, $^3J$ = 8 Hz, 2H, Ar*H*), 6.92 (s, 1H, N*H*), 6.86 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.79 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 4.66 (s, 2H, $CH_2$), 1.88-1.84 (m, 1H, CH), 1.74 (d, $^3J$ = 7 Hz, 2H, $CH_2$), 1.58 (s, 3H, $CH_3$), 1.04 (t, $^3J$ = 6 Hz, 6H, 2×$CH_3$) ppm; Anal. Calcd.for $C_{20}H_{23}ClN_2O$: C, 70.06; H, 6.76; N, 8.17. Found: C, 69.97; H, 6.81; N, 8.16.

### *4.1.29. Synthesis of1-ethyl-2,2-diisobutyl-2,3-dihydro-1H-quinazolin-4-one (2ac)*

Above mentioned general procedure was adopted for the synthesis of **2ac** by stirring **1h** (2.6 g, 10 mmol) and ethyl iodide (1.6 ml, 20 mmol) for 24 hrs. White solid; Yield 68%; m.p. 180-179 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.89 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.37-7.33 (m, 1H, Ar*H*), 6.96 (s, 1H, N*H*), 6.86 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.80 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 3.58-3.53 (m, 2H, $CH_2$), 1.90-1.86 (m, 2H, 2×CH), 1.78 (d, $^3J$= 7Hz, 4H, 2×$CH_2$), 1.14 (t,$^3J$ = 8 Hz, 3H, $CH_3$), 0.99 (d, $^3J$=8Hz, 12H, 4×$CH_3$) ppm; Anal. Calcd.for $C_{18}H_{28}N_2O$: C, 74.96; H, 9.78; N, 5.55. Found: C, 75.09; H, 9.71; N, 5.50.

### *4.1.30. Synthesis of 1-benzyl-2,2-diisobutyl-2,3-dihydro-1H-quinazolin-4-one (2ad)*

Synthesis of **2ad** was done by stirring **1h** (2.6 g, 10 mmol) with benzyl chloride (2.3 ml, 20 mmol) for 30 hrs following above mentioned general procedure. White solid; Yield 58%; m.p. 260-262 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.92 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.37-7.33 (m, 1H, Ar*H*), 7.27-7.22 (m, 2H, Ar*H*), 7.15-7.12 (m, 1H, Ar*H*), 7.11 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 2H, Ar*H*), 6.92 (s, 1H, N*H*), 6.85 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.76 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 4.68 (s, 2H, $CH_2$), 192-187 (m, 2H, 2×CH), 1.80 (d, $^3J$= 7Hz, 4H, 2×$CH_2$), 0.99 (d, $^3J$=8Hz, 12H, 4×$CH_3$) ppm; Anal. Calcd.for $C_{23}H_{30}N_2O$: C, 78.82; H, 8.63; N, 7.99. Found: C, 78.89; H, 8.6; N, 8.02.

### *4.1.31. Synthesis of 1-(2-chloro-benzyl)-2,2-diisobutyl-2,3-dihydro-1H-quinazolin-4-one (2ae)*

Compound **2ae** was synthesized by stirring **1h** (2.6 g, 10 mmol) and 2-chloro benzyl chloride (2.5 ml, 20 mmol) for 36 hrs following above mentioned general procedure. White solid; Yield 50%; m.p. 299-300 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.91 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.37-7.33 (m, 1H, Ar*H*), 7.26 (d, $^3J$ = 7 Hz, 1H, Ar*H*), 7.13-7.09 (m, 2H, Ar*H*), 7.08 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 6.90 (s, 1H, N*H*), 6.86 (t, $^3J$ = 7 Hz, 1H, Ar*H*), 6.76 (d, $^3J$ = 8 Hz, 1H, Ar*H*), 4.66 (s, 2H, $CH_2$), 191-186 (m, 2H, 2×CH), 1.79 (d, $^3J$=7Hz, 4H, 2×$CH_2$), 1.02 (d, $^3J$=8Hz, 12H, 4×$CH_3$) ppm; Anal. Calcd.for $C_{23}H_{29}ClN_2O$: C, 71.76; H, 7.59; N, 7.28. Found: C, 71.67; H, 7.63; N, 7.34.

### *4.1.32. Synthesis of 1-(4-chloro-benzyl)-2,2-diisobutyl-2,3-dihydro-1H-quinazolin-4-one (2af)*

Synthesis of compound **2af** was achieved by stirring **1h** (2.6 g, 10 mmol) and 4-chloro benzyl chloride (2.5 ml, 20 mmol) for 36 hrs utilizing above mentioned general procedure as white solid; Yield 51%; m.p. 286-287 $^0$C; $^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.90 (dd, $^3J$ = 8 Hz, $^4J$ = 1 Hz, 1H, Ar*H*), 7.36-7.32 (m, 1H, Ar*H*), 7.24 (d, $^3J$ = 8 Hz, 2H, Ar*H*), 7.08 (d, $^3J$ = 8 Hz,

2H, Ar*H*), 6.89 (s, 1H, N*H*), 6.86 (t, $^{3}J$ = 7 Hz, 1H, Ar*H*), 6.76 (d, $^{3}J$ = 8 Hz, 1H, Ar*H*), 4.66 (s, 2H, $CH_2$), 191-187 (m, 2H, 2×CH), 1.79 (d, $^{3}J$= 7Hz, 4H, 2×$CH_2$), 1.01 (d, $^{3}J$=8Hz, 12H, 4×$CH_3$) ppm; Anal. Calcd.for $C_{23}H_{29}ClN_2O$: C, 71.76; H, 7.59; N, 7.28. Found: C, 71.85; H, 7.61; N, 7.27.

**4.2. Determination of AChE and BChE inhibitory activity**

All chemicals and reagents used were of analytical grade. AChE and BChE inhibitory assay was carried out by following Ellman's methodology [44] using AChE (Electric eel type-VI-S, Sigma-Aldrich GmbH USA, code 1001596210), BChE (Equine serum Lyophilized Sigma-Aldrich GmbH USA, code 101292670) and DTNB (Sigma-Aldrich Germany, code 101261619), which produced colored product (5-thio-2-nitrobenzoate) whose concentration can be measured by the increase in absorbance at 412 nm using μQuant microplate spectrophotometer (MQX200, BioTek USA). Other reagents, like Acetylthiocholine iodide (Sigma-Aldrich UK, code 101303874), Butyrylthiocholine Iodide (Sigma-Aldrich Switzerland, code 101334643) were employed. Galantamine hydrobromide Lycoris Sp. (Sigma-Aldrich France, code G1660) and Donepezil were used as reference drugs. Stock solution of the synthesized quinazoline was prepared with 0.1 M phosphate buffer ($KH_2PO_4/K_2HPO_4$) having of pH 8.0. Appropriate amount of DTNB (Ellman's reagent), quinazoline compounds, 0.03 U/ml of enzymes (AChE and BChE) were reacted by pre-incubating at 30 °C for 10 min and then further incubating for 15 min after addition of 1mM ATCI or BTCI. Each reading was taken in triplicate and the $IC_{50}$ values were obtained by plotting sample concentration verses the inhibition.

**4.3. X-rays crystallographic study**

For X-ray crystallographic analysis**,** suitable colorless crystals of synthesized compounds **2a-af** were grown in ethyl acetate/DCM (2:1) by slow evaporation at rt. An appropriate crystal of **2j**

under monoclinic system with space groups P 21/c and crystal volume 1603.0(5) was kept at 296 K during data collection. Bruker KAPPA Apex II diffractometer with graphite-monochromatized Mo Ka radiation, $\lambda_{Mo}$ = 0.710 73 Å at 100 K was used for data collection. Data reduction was carried out on SAINT program whereas the refinement and structure solution was performed using the SHELXL-2013 program package. PLATON software was used to prepare material for publication [45-46]. Crystal structure data of compounds was deposited with Cambridge Crystallographic Data Centre (CCDC) and deposition numbers corresponding to CCDC No. 1442354, CCDC No. 1442212 and CCDC No. 1538787 were assigned to **2b**, **2j** and **2k** respectively. This data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

**4.4. Computational studies**

GOLD (Genetic Optimization for Ligand Docking) suit v5.4.1 was employed for docking studies of the synthesized compounds. 2D structures of the synthesized compounds were drawn using Marvin Sketch tool of Chemaxon [47] and energy of each compound was minimized by AM1 level of theory using ChemDraw 3D. X-ray crystallographic structures of *Tc*AChE (PDB Code 1EVE), *h*AChE (PDB Code 4EY7) and human BChE (PDB Code 1P0I) as enzyme structures. As a first step, the ability of the docking algorithm was validated to reproduce the co-crystallized poses of retrieved enzymes. For this purpose the crystal structure of donepezil was re-docked into the binding site of the AChE and the calculated root-mean-square deviation (RMSD) was found 1.29 (less than 2.0 Å, the threshold value). This yielded a good agreement between the docked and crystal structures. The binding site was identified by the with a radius of 10 Å from co-crystalized ligand. The energy minimized compounds were submitted to 10 GA runs using GOLD fitness score by applying the default docking protocol. All other docking parameters

corresponding to the software's default values were adjusted. The view of the docking results and analysis of their surface with graphical representations were done using Discovery Studio Visualizer [48].

Blood brain barrier (BBB) penetration, human intestinal absorption (HIA), mutagenic potential (AMES test and carcinogenicity were computed using online AdmetSAR server at http://lmmd.ecust.edu.cn:8000/ [49].

**Acknowledgments**

**Dr. Umer Rashid** is thankful to The Cambridge Crystallographic Data Centre (CCDC) for providing license of GOLD suit to COMSATS Institute of Information Technology. We are also thankful to Higher Education Commission (HEC) of Pakistan for financial support.

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## Highlights

- **Synthesis of** 32 **N-substituted 2,3-dihydroquinazolin-4(1H)-one derivatives**
- **Evaluated for their AChE and BChE inhibition.**
- **Excellent inhibition of both enzymes by the compounds.**
- **Molecular docking analysis**
- **In silico BBB penetration and toxicity predictions**

# Synthesis, Crystal structure determination, biological screening and docking studies of $N^1$-substituted derivatives of 2,3-dihydroquinazolin-4(*1H*)-one as inhibitors of cholinesterases

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## Graphical Abstract